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Co-variance of dissolved Fe-binding ligands with phytoplankton characteristics in the Canary Basin

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Abstract

Dissolved Fe and ligand concentrations and the Fe-binding strength of the organic ligands were measured in samples from the upper water column (150 m) of the oligotrophic waters of the Canary Basin (eastern North Atlantic Ocean). Concentrations of major nutrients, phytoplankton abundance and photosynthetic characteristics were also measured in the same samples.

The concentrations of dissolved Fe and dissolved organic ligands were low with mean values of 0.31 ± 0.18 nM Fe and 1.79 ± 0.73 nEq of M Fe(n = 47), respectively. The conditional binding constant varied between $10^{19.8}$–$10^{22.7}$ (n = 47). The largest variation with depth in the ligand concentrations (between 4.78 and 1.1 nEq of M Fe) was observed in the upper layer, above the Deep Chlorophyll Maximum (DCM located between 80 and 100 m), with high surface values in stations at 18 and 34.

At the DCM where Fe was depleted, the ligand concentrations were still relatively high showing the same trend with depth as the amount of phytoplankton cells. Here 62% of the vertical variation in ligand concentrations can be explained by parameters describing phytoplankton cell abundance or biomass and orthosilicic acid concentration, which could reflect diatom growth. Ligand concentrations below the maximum of the DCM (n = 4) showed good linear positive relationships with the total phytoplankton biomass as well as with 2 out of 4 distinguished groups of phytoplankton (*Synechococcus* and pico-eukaryote I).

In the maximum of the DCM and below this maximum the phytoplankton origin of the dissolved organic ligands of Fe is very probable. Data suggest a release of ligands by cell lysis and not by an active production. However, the origin in the surface layer is more difficult to explain. Although the amount of phytoplankton cells in the surface layer is reduced, it is still ~ 25% of the cell concentration observed in the DCM. High concentrations of organic ligands could then be a remnant of past blooms or present production under nutrient depleted conditions. Input of Sahara dust can be another source of ligands.

1. Introduction

Iron (Fe) is an essential element for phytoplankton (Sunda, 2001; Watson, 2001). The importance of Fe in marine ecology has been evident since 1988, when Martin and Fitzwater (1988) showed for the first time that Fe is, next to light, limiting phytoplankton growth in the High Nutrient Low Chlorophyll regions of the oceans (De Baar and Boyd, 2000; De Baar et al., 2005). We are only beginning to assess the role of Fe in the remaining 60% of the surface of the oceans, which hitherto was deemed to be limited only by major nutrients. Fe availability not only controls the primary productivity, but also the species

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composition and trophic structure of planktonic communities in large areas of the world oceans and seas (Martin et al., 1994; De Baar et al., 1990, 1995; Coale et al., 1996; De Baar and Boyd, 2000; Timmermans et al., 2001). Co-limitation of Fe with other parameters, such as light (Maldonado et al., 1999; Timmermans et al., 2000) and Si (Franck et al., 2000) complicates even further the relation between Fe concentration and phytoplankton growth. With regard to overall productivity of global marine ecosystems, Bruland (2003) recently has ranked Fe to be a limiting factor at least as significant as nitrogen, and definitely more severe than either phosphorus or silicon.

The biological availability of Fe for phytoplankton depends on the physical–chemical speciation of Fe, which is extremely complex and dynamic (Anderson and Morel, 1982; Hudson and Morel, 1990) but also on the species specific uptake mechanism of the phytoplankton (Hutchins et al., 1999; Maldonado and Price, 1999; Wells and Trick, 2004). Since 1994 it has been known that more than 99% of the dissolved Fe in seawater is complexed by dissolved organic ligands (Gledhill and van den Berg, 1994; Van den Berg, 1995; Wu and Luther, 1995; Rue and Bruland, 1995, 1997). These ligands highly increase Fe solubility in seawater, but also dramatically reduce the fraction of inorganic Fe (ionic Fe and Fe-hydroxides). Therefore the inorganic fraction is far too low to be the sole source of Fe for phytoplankton. By which mechanisms Fe can be released from the organic ligands to make it biologically available is still not quite clear, reduction of Fe(III) is one of the most probable possibilities. Photo-induced production of Fe(II) is the major source of Fe(II) in the marine environment (Hong and Kester, 1986; Croot et al., 2001; Rijkenberg et al., 2005) but also enzymatic reduction of FeIII on the cell surface of some phytoplankton species occurs (Jones and Morel, 1988; Maldonado and Price, 2000). Kuma et al. (1992) noticed high concentrations of Fe(II) during spring blooms in Japanese coastal waters and relates this to the release of organic compounds from phytoplankton which induces photo-reduction.

Since the organic fraction of Fe(III) is by far the largest pool, it appears as a logical supply of Fe to algal cells either directly by dissociation of the organic complex or via photo-reduction (Wells and Mayer, 1991; Miller and Kester, 1994; Hutchins et al., 1999; Kuma et al., 2000; Wells and Trick, 2004).

The question arises whether a direct or indirect mechanism exists between algae and ligand concentrations. Marine bacteria and cyanobacteria are known to excrete siderophores, i.e. a direct mechanism 'on purpose' of Fe ligand production (Reid et al., 1993; Wilhelm and Trick, 1994; Benderliev and Ivanova, 1994; Wilhelm et al., 1996; Martinez et al., 2001). It is less clear whether eukaryotic phytoplankton can excrete siderophores (Fuse et al., 1993; McCormack et al., 2003). Indications were found for a phytoplankton and/or bacterial origin of dissolved organic complexes (Van den Berg, 1995; Rue and Bruland, 1997; Boye and Van den Berg, 2000; Boye et al., 2001; Croot et al., 2001; Nakabayashi et al., 2002; Wells and Trick, 2004; Takata et al., 2004).

In this study the correlations between organic ligands and different biological parameters describing phytoplankton abundance and species composition are investigated. These correlations could indicate whether phytoplankton influences Fe solubility, either by cell lysis or excretion of ligands. Alternatively phytoplankton might be restricted to live where ligands are present and thus where Fe solubility is higher.

Samples were taken during the IRONAGES 3 cruise in the Canary Basin of the North Atlantic Ocean in autumn 2002 (Fig. 1).

2. Methods

An area, between 32–25° N, and 17–25° W was surveyed in the Canary Basin (Fig. 1) with the R.V. Pelagia in October 2002. Shallow CTD casts down to 150 m were done with an ultraclean CTD/Rosette frame attached to a kevlar hydrowire with internal signal cables. The CTD was equipped with Go–Flo water samplers, used for the trace metal clean sampling but also for nutrients and biological

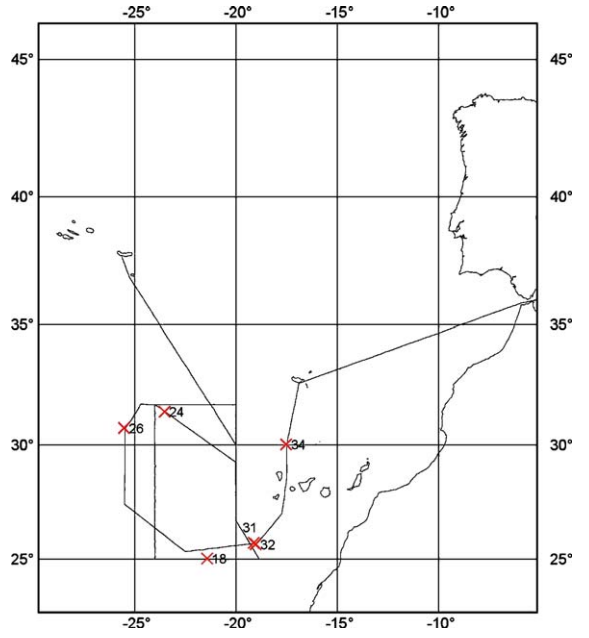

Fig. 1. Chart depicting the cruise track and numbered station locations of IRONAGES 3 cruise (4–31 October 2002).

parameters. Casts used in this study are from stations 18, 24, 26, 31, 32 and 34 (Fig. 1).

2.1. Dissolved nutrients

The samples for inorganic dissolved nutrients (nitrate, nitrite, ammonium, phosphate, orthosilicic acid) were collected in a high-density polyethylene sample bottle, filtered using a 0.2 μm acrodisc filter and stored in the dark at 4 °C in a polyethylene cup. All samples were analysed within 8 h using a Technicon TrAAcs 800 autoanalyzer. The different nutrients were measured colorimetrical as described by Grasshoff (1983).

2.2. Dissolved iron

All sample handling was performed inside an overpressurised class 100 clean air container on board. Sampling was done using modified Teflon coated PVC General Oceanics Go–Flo bottles. After recovery of the bottles they were mounted inside the storage cabinets, inside the clean air container. Filtered nitrogen was used to filter the water from the bottles over a 0.2 μm filtration cartridge (Sartobran–P capsules, Sartorius).

Samples for dissolved iron analysis were acidified to pH 2.0 using 1 mL of ultrapure® hydrochloric acid (HCl, Merck) per 1 L of sample for at least 24 h before analysis.

Dissolved iron concentrations were measured on board according to a chemo luminescence method adapted from Obata et al. (1993) (see Bucciarelli et al., 2001; Sarthou et al., 2003). The detection limit, defined as three times the standard deviation of the blank, was equal to 0.027± 0.017 nM. A complete dataset of dissolved Fe is presented elsewhere (Sarthou et al., submitted for publication).

2.3. Dissolved organic ligands

Samples were treated as described for the analysis of dissolved Fe, with the exception that the samples were not acidified. Most samples were analysed within 2 days after sampling, being stored either in the dark at 4 °C, or frozen. All samples were analysed on board except station 34, that was analysed at the home laboratory within 1 month after sampling. Determination of the organic speciation of iron in seawater water was performed using competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV). 2-(2-Thiazolylazo)-p-cresol (TAC) (Aldrich, used as received) was used as competing ligand (Croot and Johansson, 2000). All solutions were prepared using 18.2 MΩ nanopure water. The equipment consisted of a μAutolab voltammeter (Ecochemie, Netherlands), a static mercury drop electrode (Metrohm Model VA663), a double-junction Ag/saturated AgCl reference electrode with a salt bridge containing 3 M HCl and a counter electrode of glassy carbon. The titration was performed using 0.01 M stock solution of TAC in three time quartz-distilled (3× QD) methanol, 1 M boric acid (Suprapur, Merck) in 0.3 M ammonia (Suprapur, Merck) (extra cleaning by the addition of TAC after which TAC and $Fe(TAC)_2$ was removed with a C18 SepPak column) to buffer the samples to a pH of 8.05 and a 10^{-6} M Fe(III) stock solution acidified with 0.012 M HCl (3× QD). Aliquots of 15 ml were spiked with Fe(III) until final concentrations between 0 and 8 nM and allowed to equilibrate overnight (>15 h) with 5 mM borate buffer and 10 μM TAC. The concentration $Fe(TAC)_2$ in the samples was measured using the following procedure: i) removal of oxygen from the samples for 200 s with dry nitrogen gas, a fresh Hg drop was formed at the end of the purging step, ii) a deposition potential of −0.40 V was applied for 30–60 s according to the sample measured, the solution was stirred to facilitate the adsorption of the $Fe(TAC)_2$ to the Hg drop, iii) at the end of the adsorption period the stirrer was stopped and the potential was scanned using the differential pulse method from −0.40 to −0.90 V at 19.5 mV s^{-1} and the stripping current from the adsorbed $Fe(TAC)_2$ recorded. The detection limit was 0.1 nM $Fe(TAC)_2$ (three times the standard deviation of the reagents blank at 60 s deposition), the reproducibility of the titration was less than 10% (n=4).

The principle of measuring the binding characteristics of organic ligands with Fe is extensively described by Gledhill and van den Berg (1994), and by Croot and Johansson (2000). A known organic ligand is added to the sample, in this case TAC. This ligand is strong enough to compete with the natural organic ligands for reversibly bound Fe. After equilibration overnight equilibrium exists between TAC and the natural ligands, according to:

$$K'_{\mathrm{TAC}}{}^{*}[\mathrm{TAC}]^2/K'_{\mathrm{ligands}}{}^{*}[\mathrm{L}] = [\mathrm{Fe(TAC)_2}]/[\mathrm{FeL}]$$

where K' is the conditional stability constant of Fe with the ligands, (either TAC or the natural organic ligands (L)) and [L] and [TAC] are the concentrations of free (not Fe bound) ligands. The $[Fe(TAC)_2]$ and [FeL] are the concentrations of both Fe complexes.

The concentration $[Fe(TAC)_2]$ is measured by CSV. Since the added concentration of TAC and its binding strength with Fe are known, the product of the conditional stability constant and the free natural ligand concentration are known after one measurement. In order to estimate these parameters separately, a series of ten sub samples with increasing Fe concentrations is made. After equilibration overnight the empty binding sites of the natural

organic ligands are filled with Fe in the sub samples with the highest Fe additions. Then a linear relation between the $Fe(TAC)_2$ concentrations and the Fe additions is found. When the natural organic ligands are not yet filled a non-linear response between added Fe and the $Fe(TAC)_2$ concentrations is found. It is possible to estimate L and K' of the natural ligands from such a curved response (Van den Berg, 1982) using the non-linear regression of the Langmuir isotherm (Gerringa et al., 1995). The results, including the 95% confidence interval of the non-linear regression on logK' and [L] are presented in Table 1, large confidence intervals indicated with an asterisk are due to noise caused by problems with the electrode. The membrane regulating the mercury flow was torn during the cruise, and this caused from time to time a fracture as fine as a hair in the upper part of the glass of the capillary, there where the capillary has widened into a mercury reservoir. A crack here did permit measurements to continue, however the noise increased. When this happened during a titration, continuing was a better option than losing the sample and the capillary was changed after finishing the titration.

2.4. Phytoplankton characteristics

The composition of the phytoplankton community into two species and two groups of phytoplankton was characterised based on the cellular bio-optical properties (size, scatter and chlorophyll fluorescence) of algal cells by applying flow cytometry (Veldhuis and Kraay, 2000, 2004). Small volume samples (2 mL) were taken for detailed analysis of the pico-phytoplankton community (<20 μm). The instrument applied in the single cell analysis of the phytoplankton community was a bench top flow cytometer (Coulter XL-MCL). This instrument is equipped with a 15 mW laser (488 nm excitation) and emission bands in the orange (FL2: 575±20 nm) to detect the presence of phycoerythrin and red (FL3>630 nm) to collect the chlorophyll fluorescence signal. In addition forward light scatter is collected as a fourth parameter. Phytoplankton is distinguished from other particles based on their chlorophyll fluorescence, which is collected in the red detector (hereafter called Fl). Samples were analysed freshly, shortly after sampling. Typical volume analysed was 1450 μl, in some cases when numbers were really low (<500 counts) this volume was doubled.

Samples were analyzed based on the presence of clearly distinguishable groups. The dominant species were *Prochlorococcus* spp., *Synechococcus* (based on the presence of phycoerythrin) and two different groups of pico-eukaryotes. Fractionated filtration showed that more than 95% of the phytoplankton biomass was smaller than 2 μm. The product of the chlorophyll auto fluorescence (Fl) and cell number provided an estimate for the chlorophyll specific biomass of each species or group.

A PAM fluorometer (Pulse Amplitude Modulated-CONTROL Universal Control Unit, WATER-mode, Walz, Germany) was used to determine Fo (auto fluorescence), Fm (maximum fluorescence) and Fv/Fm (Fv=Fm−Fo, photosynthetic efficiency) in dark adapted phytoplankton samples. Sub maximal values (<0.3) of Fv/Fm are strong evidence of physiological stress (Geider and LaRoche, 1994).

The samples for Chlorophyll-*a* (Chl-*a*) (typically 1.5 L) were collected on a GF/F filter, after which they were stored at −80 °C. All samples were analysed during the cruise as described by Veldhuis and Kraay (2004).

2.5. Statistical methods

The samples were subjected to automated backward stepwise multiple regression computer package SYSTAT (version 10, 2000), using the default values for fitting, concerning the minimum tolerance for entry and/or removal being 0.015 (as alpha to enter and as alpha to remove meaning 85% nominal significance level). The values of the different parameters were entered in their relative units and cell numbers in cells ml^{-1}. Calculated population specific biomass values were normalised to values ranging between 3 and 150. Both the total ligand concentration and the excess of empty ligand sites, being the total ligand concentration minus the dissolved Fe concentration, were used as dependant variables. The ligand concentration was eventually preferred, giving better relationships with the other variables. First all measured variables were tested for a relationship with the ligand concentration, both by single parameter regression and with SYSTAT applying multiple regression.

Only independent variables were tested. For example density was chosen in favour of depth, salinity and temperature, all being interrelated with each other. Similarly, biomass (product of cell abundance and Fl) was not used in the same regression as fluorescence (Fl) and cell counts. Per plankton group, distinguished by flow cytometry, either Fl as well as the amount of cells was used in multiple regression analysis or the biomass alone. Nitrate, nitrite, ammonium and phosphate did not show any relation with the ligand concentration and only orthosilic acid was used for further statistical analysis. We treated the data set as a whole (all data, $n=47$), and as a data set of samples between 70–80 and 150 m (deep samples, $n=26$), defined by the presence of phytoplankton (>5000 cells ml^{-1}). This latter group of deep samples was in a later stage divided into three subgroups according to the relations between ligand concentration and phytoplankton

Table 1
Dissolved Fe (nM) and the characteristics of the dissolved organic ligands, the ligand concentration (L) and the conditional stability constant K'. The surface mixed layer (SML), above the maximum of the deep chlorophyll maximum (MDCM), the MDCM and below the MDCM are indicated

Stations	Depth (m)	Fe (nM)	Lt (neq of M Fe)	95% confidence interval	logK'	95% confidence interval	Characterization water layer
18	10.0	0.63	3.96	0.60	19.8	0.22	SML
18	25.7	0.67	1.71	016	20.53	0.27	
18	60.4	0.31	1.11	0.06	20.59	0.22	
18	80.0	0.14	1.47	0.19	20.61	0.32	above MDCM
18	89.2	0.13	1.51	0.28	20.05	0.4	MDCM
18	100.7	0.19	1.03	0.24	20.33	0.65	
18	125.2	0.23	1.37	0.36	21.16	0.96*	
18	150.7	0.43	0.83	0.55	20.48	2.08*	
24	10.7	0.28	1.28	0.22	19.88	0.3	SML
24	26.2	0.18	2.02	0.19	20.26	0.28	
24	50.8	0.10	2.54	0.53	19.79	0.44	
24	70.3	0.09	1.83	0.56*	19.89	0.53*	above MDCM
24	80.2	0.07	1.01	0.11	20.92	0.19	MDCM
24	91.3	0.06	1.7	0.4	19.79	0.31	
24	100.2	0.08	1.13	0.11	20.68	0.35	
24	150.2	0.16	2.03	0.56*	20.27	0.52*	
26	26.6	0.35	2.06	0.16	20.33	0.2	SML
26	70.1	0.18	2.38	0.34	20.14	0.25	
26	80.7	0.20	1.86	0.44	19.68	0.35	above MDCM
26	90.0	0.22	1.38	0.3	19.68	0.33	MDCM
26	99.6	0.18	1.39	0.32	19.83	0.43	
26	152.2	0.27	1.13	0.21	20.14	0.38	below MDCM
31	10.9	0.45	2.07	0.68*	19.78	0.52*	SML
31	26.0	0.53	1.18	0.36*	21.66	0.61*	
31	50.3	0.43	2.11	0.45	20.33	0.45	
31	70.1	0.42	1.53	0.29	20.24	0.5	above MDCM
31	80.2	0.39	1.52	0.22	21.98	0.33	
31	90.1	0.39	1.13	0.36	21.64	0.53	MDCM
31	99.5	0.35	1.97	0.3	22.06	0.32	
31	151.4	0.43	1.58	0.43	19.87	0.43	below MDCM
32	9.8	0.36	1.68	0.24	20.18	0.32	SML
32	25.7	0.44	1.35	0.2*	22.09	1.5*	
32	50.6	0.39	2.08	0.39*	20.6	0.49*	
32	70.5	0.42	1.81	0.3	19.93	0.3	
32	80.2	0.42	1.89	0.31	19.99	0.28	
32	91.6	0.42	2.18	0.12	20.42	0.13	above MDCM
32	101.6	0.33	2.19	0.24	20.17	0.23	MDCM
32	126.2	0.30	1.73	0.29	20.01	0.3	
32	151.8	0.36	1.63	0.22	20.13	0.27	below MDCM

Table 1 (*continued*)

Stations	Depth (m)	Fe (nM)	Lt (neq of M Fe)	95% confidence interval	logK'	95% confidence interval	Characterization water layer
34	10.6	0.13	4.78	1.9	21.06	0.36	SML
34	23.9	0.14	3.11	1.4	21.19	0.45	
34	50.9	0.21	1.49	0.27	21.62	0.23	
34	61.1	0.15	2.52	0.38	21.92	0.2	
34	70.8	0.11	2.03	0.27	22.67	0.3	
34	80.7	0.10	2.75	0.58	21.22	0.21	
34	90.1	0.10	1.17	0.16	21.98	0.33	MDCM
34	101.2	0.12	1.56	0.35	21.96	0.48	
34	125.7	0.15	1.1	0.3	21.77	0.6	
34	150.3	0.24	1.25	0.19	21.95	0.35	below MDCM

* Large errors indicated with an asterisk are due to noise caused by problems with the membrane regulating the mercury flow (see Method section).

abundance shown in Fig. 6A and Table 4. One group consists of samples taken at the real maximum of phytoplankton abundance (more or less the samples with more than 10,000 cells ml^{-1}), hereafter called: MDCM (maximum deep chlorophyll maximum; $n=16$) and two subgroups at the borders of the maximum phytoplankton abundance (more or less samples with phytoplankton abundance between 5000 and 10,000 cells ml^{-1}), thus above ($n=6$) and below ($n=4$) the MDCM (Table 1 and Fig. 5). The motivation of the division into three groups is more extensively explained in the result and discussion sections. Multiple regression could be applied to the

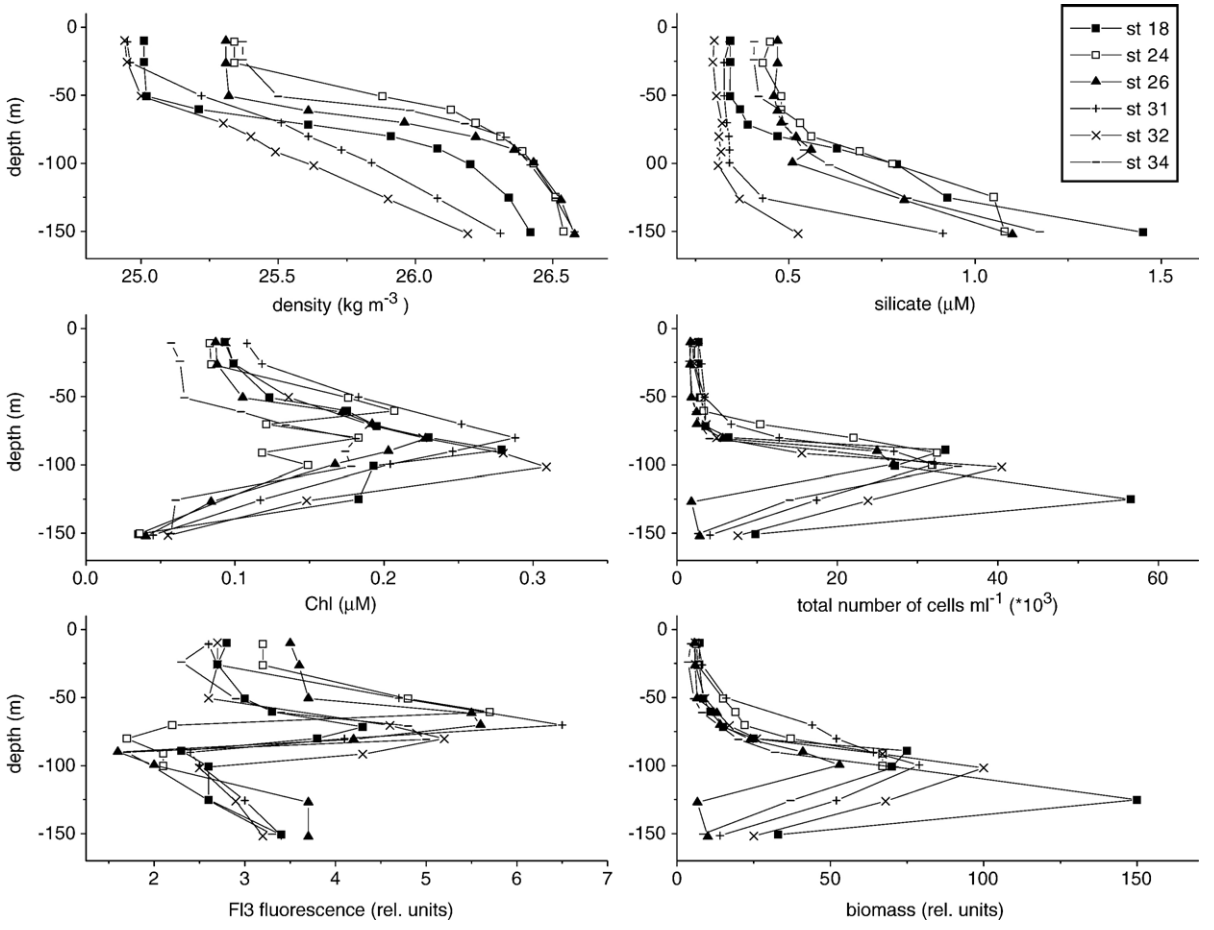

Fig. 2. Vertical profiles of A: density (kg m^{-3}), B: silicate (µM), C: Chl-*a* (µM), D: the total cell numbers (*10^3), E: total chlorophyll autofluorescence (Fl) (relative arbitrary units) and F: total biomass (relative arbitrary units) of the stations 18, 24, 26, 31, 32 and 34 as shown in Fig. 1.

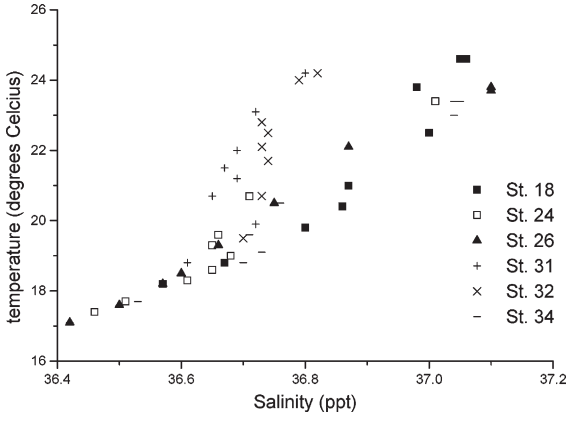

Fig. 3. Property–property plot of temperature (°C) versus salinity (ppt) of the stations 18, 24, 26, 31, 32 and 34 (Fig. 1).

samples in the MDCM, single linear regression was applied to the MDCM samples ($n=16$) and to the samples above ($n=6$) and below the MDCM ($n=4$).

3. Results

The surface mixed layer (SML) extends to 25 m in stations 24, 31 and 34 and to 50 m in stations 18, 26 and 32 (Fig. 2), with a stable stratification deeper in the water column and thus generally a low turbulent exchange of matter. According to the property–property plot of temperature and salinity the upper layer of stations 31 and 32 consists of slightly different water from the other stations (Fig. 3).

In general dissolved inorganic nutrients start to increase around the DCM. Station 18 is the most nutrient-rich followed by 34, 24 and 26, respectively (Table 2). Stations 32 and 31 are the poorest in nutrients.

In the area covered in our survey, the highest phytoplankton standing stock (56 000 cells ml^{-1}) is found in station 18 where the highest nutrient concentrations are found (Fig. 2 and Table 2).

The Chl-*a* values show a typical pattern of surface minimum (ranging from 0.05 to 0.1 μg/l) and a gradual increase to peak values (ranging from 0.15 to 0.3 μg/l) at a depth of 80 to 100 m (Fig. 2). Values drop rapidly with depth below the DCM and phytoplankton is virtually absent below 150 m. The phytoplankton community is dominated by pico-phytoplankton species such as *Synechococcus*, *Prochlorococcus*, and two groups of pico-eukaryotes (groups I and II; Fig. 4). This pattern is consistent for all 6 stations examined. Vertical patterns of numerical distributions and cellular chlorophyll fluorescence are identical for all 6 stations, although they differ occasionally in absolute values.

A slight difference in depth between the biological maxima can be distinguished, Chl-*a* maxima of the stations considered are found at 80 m depth, whereas maximum values of chlorophyll fluorescence are present at 70 to 80 m depth, total cells maximum is slightly deeper than 100 m, resulting in a total biomass maximum (A*FL) at 100 m (Figs. 2 and 4). Also the biomass maximum of the different species and groups recognised have different mean depths, for *Synechococcus* at depths above 100 m, for pico-eukaryote I (this maximum is difficult to pinpoint) at 100m, for pico-eukaryote II and *Prochlorococcus* deeper than 100 m (Fig. 4).

For stations presented here, most of the profiles show a maximum of dissolved Fe in the surface mixed layer ranging from 0.18 nM to 0.67 nM (stations 18, 24, 26, 31 and 32), which can reflect the atmospheric inputs in this region (Table 1). The dissolved Fe concentrations agree well with values previously observed in this region (Vink and Measures, 2001; Bowie et al., 2003; Sarthou et al., 2003). The lowest concentrations of dissolved Fe (between 0.06 nM, station 24, and 0.35 nM, station 31) coincide with the maximum of Chl-*a* and increase again with depth. At 150 m, values range from 0.16 nM (station 24) to 0.43 nM (stations 18 and 31).

The dissolved organic ligands are always in excess of dissolved Fe. The conditional stability constant of the dissolved organic Fe complex varies as found by others between $10^{19.5}$ and 10^{22} (Gledhill and van den Berg, 1994; Rue and Bruland, 1995, 1997; Nolting et al., 1998; Boye et al., 2005) (Table 1). No real trend can be distinguished in

Table 2
Nutrient concentrations in μM in the surface mixed layer (SML), the deep chlorophyll maximum (DCM) and at 150 m depth of the stations, together with the depth of the DCM in m. *N*=sum of nitrate, nitrite and ammonium

St. #	Si SML	N SML	P SML	Depth DCM	Si DCM	N DCM	P DCM	Si 150 m	N 150 m	P 150 m
18	0.35	0.15	0.02	90	0.63	0.36	0.04	1.45	5.15	0.30
24	0.44	0.21	0.02	60	0.48	0.25	0.02	1.08	2.95	0.16
26	0.47	0.16	0.01	80	0.56	0.19	0.01	1.10	3.53	0.19
31	0.33	0.14	0.03	100	0.36	0.39	0.05	0.91	2.60	0.17
32	0.30	0.20	0.02	100	0.31	0.25	0.03	0.53	1.29	0.09
34	0.41	0.17	0.01	80	0.51	0.12	0.01	1.19	3.92	0.22

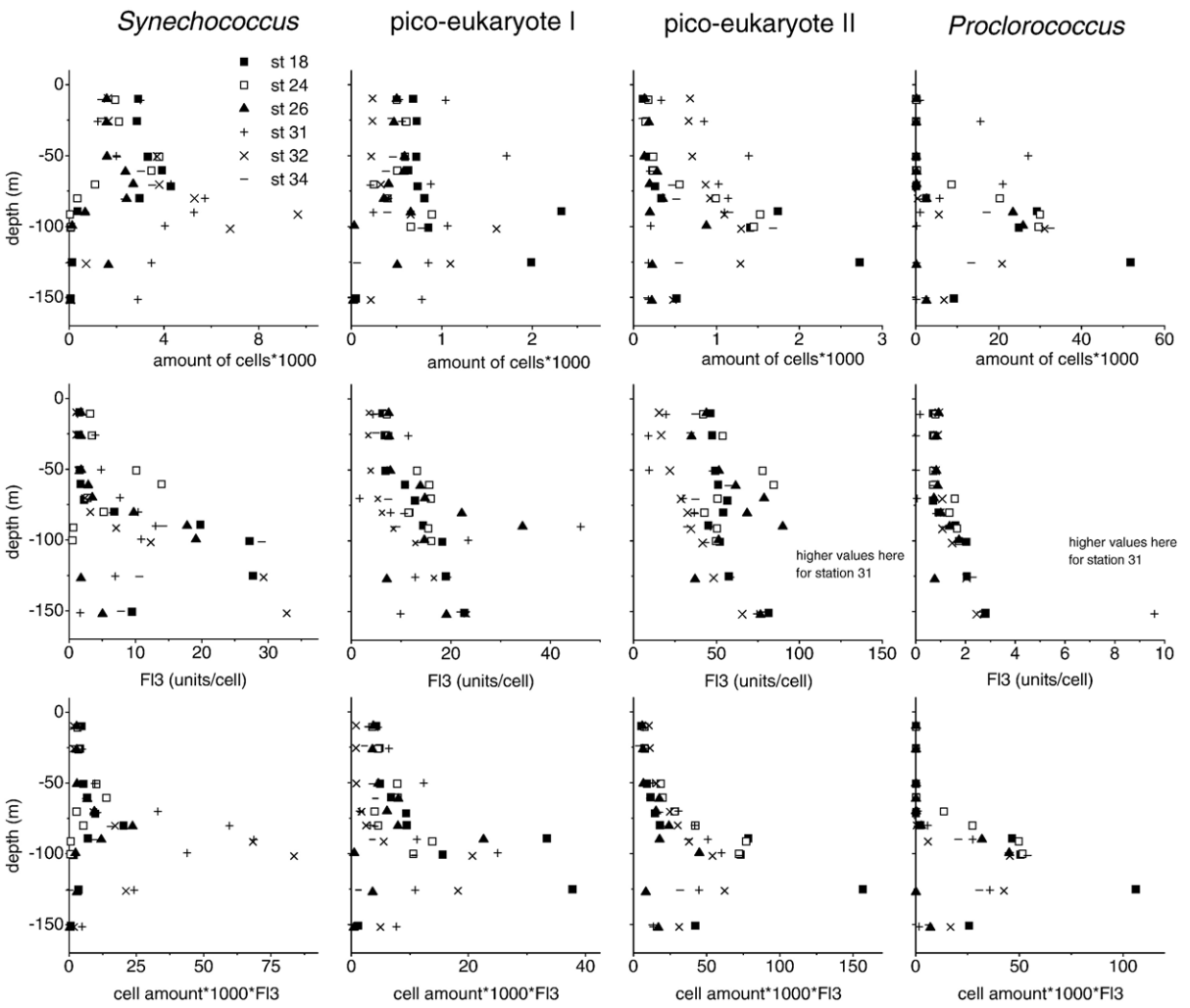

Fig. 4. Vertical profiles of cell numbers ($*10^3*mL^{-1}$), chlorophyll autofluorescence (Fl) (relative arbitrary units) and biomass (relative arbitrary units) of phytoplankton specie *Synechococcus* (A), two different pico-eukaryote groups: pico-eukaryote I (B) and pico-eukaryote II (C) and the species *Prochlorococcus* spp. (D) with depth of the stations 18, 24, 26, 31, 32 and 34 (Fig. 1).

K', but values at station 34, near the Canary Islands, are higher than elsewhere (10^{21} to $10^{22.6}$). Two stations show high ligand concentrations in the surface layer (3 to 4 nEq of M Fe at stations 18 and 34). In other stations the profiles of Fe and L are rather straight with highest variation in the upper water 70 to 80 m.

A slight maximum in the dissolved organic ligand concentrations is observed near the DCM round 80 to 120 m depth (Fig. 5). It is this maximum, visible in all stations except station 26, that suggests a possible relationship with biological parameters. Although the phytoplankton biomass is relatively low, differences in numbers and Chl-*a* content with respect to water column depth as discussed above, give rise to the question whether it is possible to establish relationships between the ligand concentration and the different biological parameters. Therefore samples with elevated phytoplankton (>5000 cells. ml^{-1}) are selected, below 70–80 m depth, including the DCM. Multiple stepwise regressions are used to estimate a possible relationship between the ligand concentration and the other parameters. In the surface layer (<5000 cells. ml^{-1}) no significant relations with L were found.

When the amount of cells and biomass are considered two clusters of data points become visible (Fig. 6A). Two linear relationships between the dissolved organic ligand concentration and the total amount of cells are present, one coinciding with samples containing the maximum numbers of cells per station, more or less having more than 10,000 cells ml^{-1} (closed squares in Fig. 6A, MDCM) and one with samples with cell amounts more or less between 5000 and 10,000 cells ml^{-1} (open symbols and crosses in Fig. 6A, see also Table 1 and Fig. 5). The

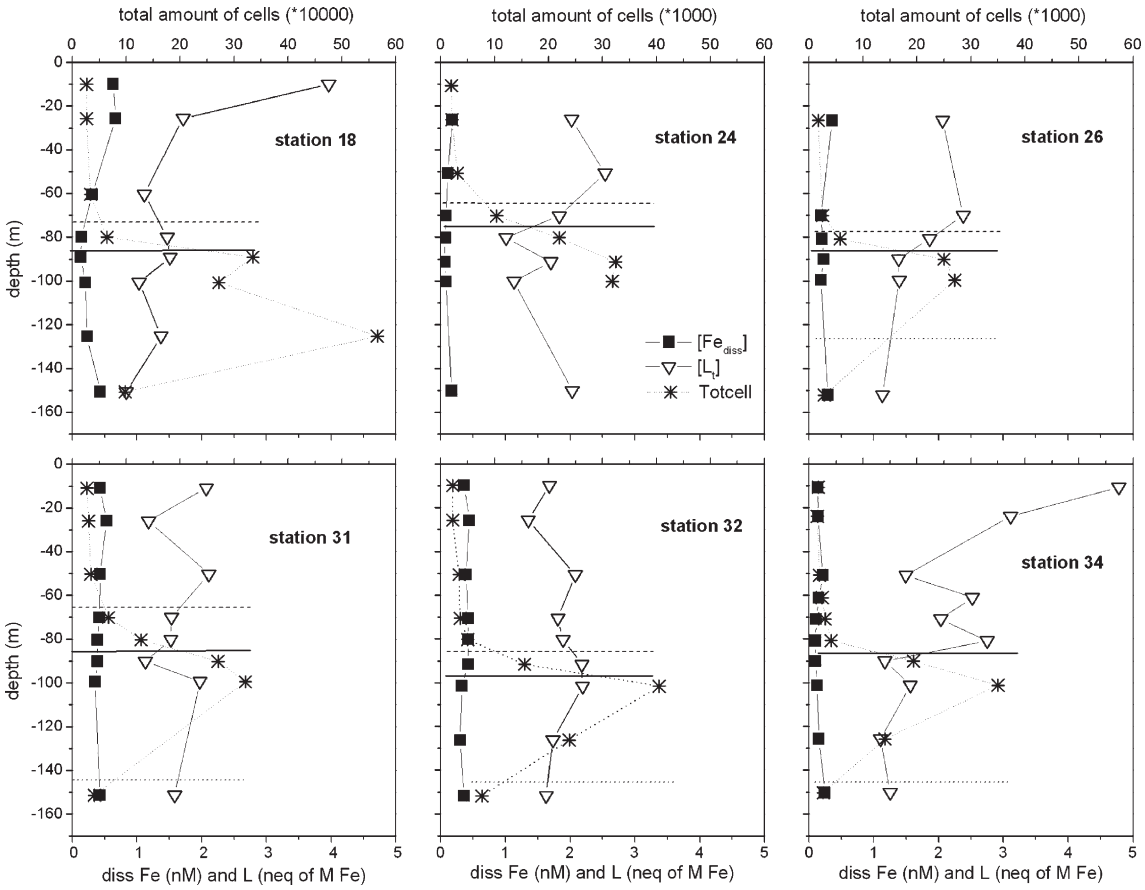

Fig. 5. Dissolved Fe (nM), dissolved organic ligands (nEq of M Fe) and total amount of phytoplankton cells ($*10^3*mL^{-1}$) versus depth of all stations. A dashed horizontal line separates "surface" samples from samples above the MDCM, a solid line separates samples from above the MDCM from samples in the MDCM and a dotted line separates the MDCM from samples below the MDCM (also indicated in Table 1).

slopes of these relationships differ considerably being 15.2 ($n=26$, $R^2=0.55$) for samples in the MDCM, 10.4 for the samples just above and below the MDCM ($n=10$, $R^2=0.55$, not shown in Table 4) and 7.6 ($R^2=0.66$) for the samples below the MDCM ($n=4$) (Table 4). Although the water above and below the MDCM have in common that conditions are limiting the growth of the phytoplankton, the causes for growth limitation are different, being nutrient limitation and possibly due to grazing pressure above the MDCM and light limitation below the MDCM. They are treated as separate groups in the following.

In the MDCM 70% of the variation in L can be explained as shown in Eq. (1) in Table 3, whereas the error of the estimated L is rather low (0.24 nEq of M Fe). This relationship is negatively related to the silicate concentration and the biomass of pico-eukaryote I. It is positively related to the amount of phytoplankton cells expressed as total amount of cells and their fluorescence (Fl) (Figs. 5 and 6). Since a negative relationship between L and the biomass of pico-eukaryote I is not logic as can be deduced from Fig. 6F, the multiple regression was repeated without the biomass of pico-eukaryote I (Eq. (2) in Table 3), resulting in a lesser fit, but slightly better significances of the explanatory variables. Single linear relationships exist for the ligand concentration with the Si concentration for the samples below the MDCM, with the total amount of cells and with the biomasses of 2 out of 4 distinguished groups of phytoplankton, *Synechococcus* and pico-eukaryote I, respectively (Table 4).

4. Discussion

The general picture arising from the survey in the Canary Basin is an oligotrophic system with low nutrient concentrations, low phytoplankton standing stocks, and relatively low Fe concentrations (Figs. 2 and 4 and Tables 1and 2). In the surface mixed layer in which nutrients are depleted, the numbers of plankton cells are relatively low, whereas the ligand concentrations are high and vary considerably (Figs. 2 and 5 and Table 1). The input of

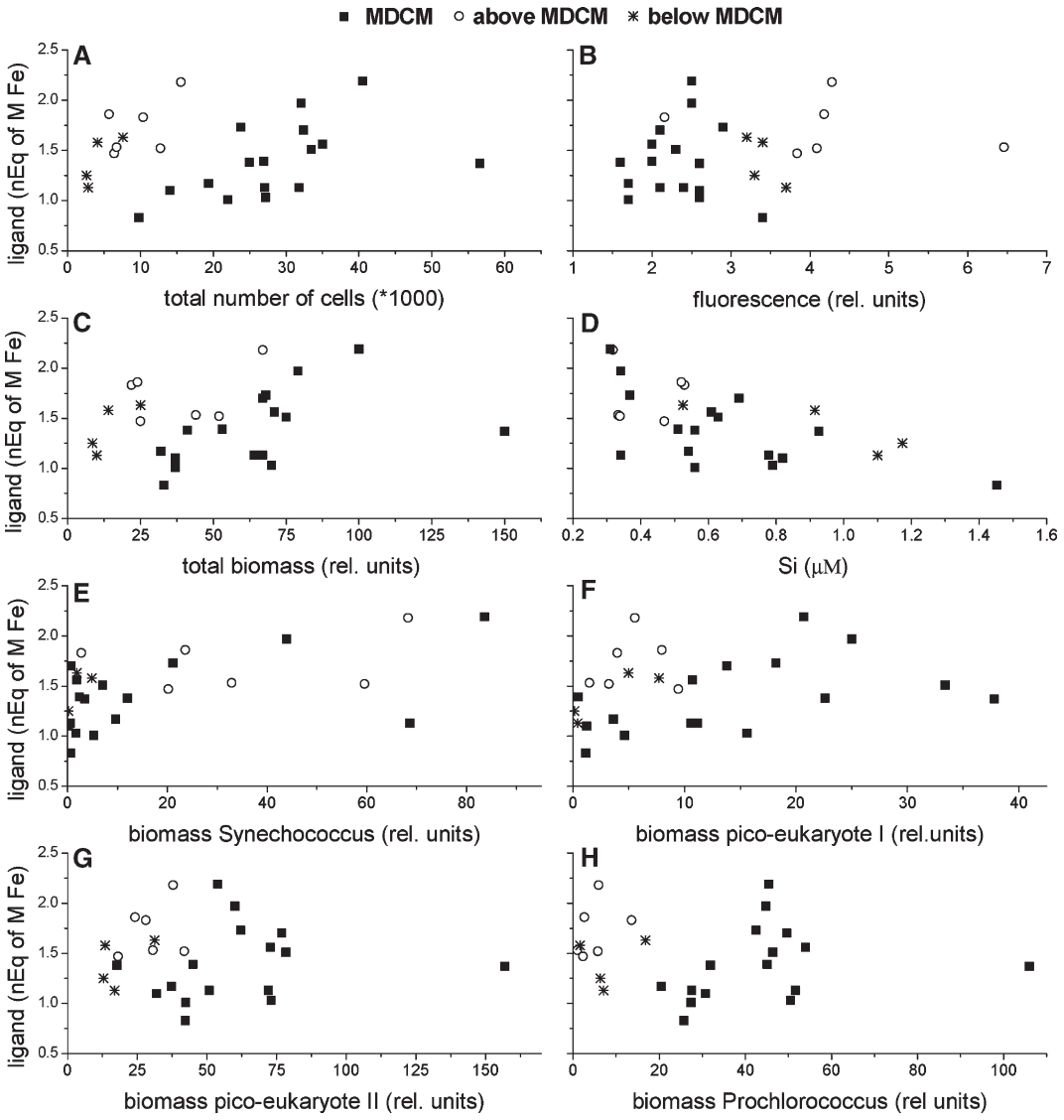

Fig. 6. The dissolved organic ligand concentration (nEq of M Fe) of the samples from the MDCM (closed squares) above the MDCM (open circles) and below the MDCM (asterisks) versus: (A) the total amount of phytoplankton cells ($*10^3$*mL^{-1}), (B) the total fluorescence (relative arbitrary units), (C) total biomass, (relative arbitrary units), (D) the silicate concentration (μM), (E,F,G,H) biomass of *Synechococcus*, pico-eukaryotes I and II, and *Prochlorococcus* spp. (relative arbitrary units,) respectively.

ligands by Sahara dust can be an explanation for the high surface concentrations (Nakabayashi et al., 2002; Kramer et al., 2004). Dust as a source of dissolved organic ligands was already suggested by Saydam and Senyuva (2002) and Visser et al. (2003). The influence of atmospheric input of Fe can not be denied in this area and will certainly be of some importance although high surface ligand concentrations in stations 18 and 34 do not coincide with the NW part of the area where the highest Al concentrations, used as tracer for dust input, were observed (Kramer et al., 2004). A rapid increase in organic Fe-binding ligands after iron enrichment suggested a fast response of the microbial community during Ironex-II (Rue and Bruland, 1997).

The amount of phytoplankton cells in the surface layer is low, however it still amounts to 25% of the values observed in the MDCM for two out of four distinguished groups, *Synechococcus* and the pico-eukaryote I. Since exactly those two groups of phytoplankton in the MDCM show positive linear relationships with the ligand concentration (Table 4, Fig. 4A,B) they also might be responsible for part of the production of the ligands in the surface layer, although no relation was found by applying regression analysis. *Synechococcus* is known to produce

Table 3
Statistical information on the ligand concentration Lt and relations between Lt and other parameters (Eqs. (1) and (2)) at the deep chlorophyll maximum. The division of the samples in maximum deep chlorophyll maximum (MDCM) above and below the MDCM is shown in Fig. 5 and Table 1

Mean values of Lt and multiple linear relations of Lt (neq of M Fe) with other parameters.	*N*	R^2	St error of estimated L
All data			
Mean [Lt]=1.79 neq of M Fe, SD from average=0.73	47		
Shallow samples (<5000 cells ml^{-1})			
Mean [Lt]=2.12 neq of M Fe, SD from average=0.9	21		
Above MDCM (>5000 cells ml^{-1})			
Mean [Lt]=1.7 neq of M Fe, SD from average=0.3	6		
MDCM (>10,000 cells ml^{-1})			
Mean [Lt]=1.4 neq of M Fe, SD from average=0.37	16		
Eq. (1): [Lt]=0.413–0.58 Si+0.036 Totcell+0.363 TotFl−0.009C*Fl	16	0.700	0.24
Eq. (2): [Lt]=0.98–0.9 Si+0.013 Totcell+0.261 TotFl	16	0.621	0.26
Below MDCM (>5000 and <10,000 cells ml^{-1})			
Mean [Lt]=1.4 neq of M Fe, SD from average=0.25	4		

N is amount of samples, R^2=least square root of the fit. Parameters are used in their normal unities, the number of cells is divided by 1000 before used in the regression procedure. Totcell=number of all cells, TotFl=fluorescence of all cells, Totcell*Fl, C*Fl is the product of number of cells (again first divided by 1000) and the fluorescence (Fl) of all cells and pico-eukaryote I, respectively.
The significance (*P* value) of the independent contribution of the variation of L for the constants and parameters of Eq. (1) are 0.357, 0.075, 0.037, 0.049, 0.138 and for Eq. (2) are 0.028. 0.006, 0.055, 0.124, respectively.

siderophores (Wilhelm et al., 1996, 1998). Although we have no information on primary production to relate with the ligand concentration, productivity is always high in surface water (Sathyendranath et al., 1995). Since in surface waters a relative high percentage of phytoplankton cells are non-viable (Veldhuis et al., 2001), organic compounds leaking out of these dying cells may act as ligands. The origin of the organic ligands can not be deduced from our data, atmospheric dust and lysis of phytoplankton cells as well as siderophore production are all possible sources in our research area.

Table 4
Linear relations of the concentration of the dissolve organic ligands (neq of M Fe) with the Si concentration (μM), total cell amounts ml^{-1} (Totcell) and total biomass (TotFl) the biomass of *Synechococcus* (AFl), and pico-eukaryote I (BFl) (relative arbitrary units)

Layer and parameter identification	Linear relations of Lt (neq of M Fe) with single other parameters.	*N*	R^2
Si			
Below MDCM	[Lt]=2.3−0.99 [Si]	4	0.6970
Total cell amounts			
MDCM	[Lt]=5.6+15.2 Totcell	16	0.5429
Below MDCM	[Lt]=−6.4+7.6 Totcell	4	0.6583
Biomass			
Below MDCM	[Lt]=18.6+23.55 TotFl	4	0.6488
	[Lt]=−7.6+6.8 AFl	4	0.5768
	[Lt]=−15.5+13.5 BFl	4	0.8060

The samples with cell amounts >5000 ml^{-1} above the MDCM did not show any relation (R^2<0.5) with any other parameter. The data from the MDCM and from the samples below the MDCM do suggest a relation between phytoplankton the concentration of dissolved organic ligands as shown in Fig. 6, and Tables 3 and 4. The single linear relations depict the influence on ligand concentrations of only one parameter, whereas the multiple regression tries to combine the contribution of each parameter into one single relation (Tables 3 and 4). The relation from the multiple regressions between the ligand concentration and the total cells and chlorophyll fluorescence show a relatively small standard error of estimated L of 0.24 to 0.26 nEq of M Fe. This error is similar to the confidence interval of the measured values of L (Table 1). Although Eq. (1) (Table 3) gives the best fit, it is not a realistic relationship since the biomass of pico-eukaryote I relates negatively with the ligand concentrations whereas Fig. 6F and Table 4 show that the relationship is positive, thus we focus in the following on Eq. (2) of Table 3.

The constant in the equations can be seen as that fraction of the ligand concentration that is not influenced and or determined by the parameters considered. The constant differs considerably in different relations in Tables 3 and 4 since the single parameter regressions do not reflect the potential influence of other parameters.

The amount of cells and the chlorophyll fluorescence or the biomass are direct biological parameters relating to the ligand concentration at and below the MDCM. Moreover, the Si concentration, a parameter in the multiple linear regression and in the single parameter regressions, reflects another biological parameter when taken as a result of diatom growth (Tables 3 and 4). The coefficients of the Si concentration in Eq. (2) (Table 3) and of samples below the MDCM (Table 4) are almost equal (0.9 and 0.99 respectively) indicating a constant influence with depth. However, the question arises whether there are enough diatoms to guarantee this indirect biological relation. Analysis of the plant pigment did not show typical diatom pigments (data not shown), so the amount of diatoms must be very low. This observation is in agreement with earlier studies in the same region and same season confirming low abundance of diatoms (Veldhuis and Kraay, 2004). It is also known that large diatom cells were not present because they cannot grow at such low Fe concentrations (Sunda et al., 1991; Sunda and Huntsman, 1995; Timmermans et al., 2004; Sarthou et al., 2005). On the one hand Si is the only nutrient that is related to the ligand concentration indicating that diatom growth is an important factor; on the other hand diatoms are hardly present. Diatoms are thought to produce organic ligands as suggested by Trick et al. (1983), Croot et al. (2001) and recently by Armbrust et al. (2004). The relation in this survey between Si and the ligand concentration suggests that ligands produced by the diatoms during spring bloom, with sufficient nutrient concentrations (including orthosilic acid), are still present. Since ligands are found in all marine waters at least some fraction must be very resistant to degradation.

The different coefficients in the single parameter relation of L with the total amount of cells warrant the division of the samples in different groups (MDCM and below the MDCM) (Fig. 6A and Table 4). This difference in coefficient of a factor two (15.2 versus 7.6) suggests that cells living below the MDCM, where light is expected to become limiting, excrete or loose more ligands per cell than cells living in the MDCM. When we assume that the amount of cells reflects the conditions for growth, non-optimal conditions can cause an increase in ligand concentration. Fv/Fm values indicate stress, including Fe limitation. This ratio does not show any relation with the ligand concentration, indicating that an increase in ligands is not a result of Fe limitation. Moreover, Fe concentrations are relatively high below the MDCM compared to the MDCM. The ligands were not produced to alleviate Fe limitation and an inadvertent release of ligands causes the relation between the ligand concentration and biomass of phytoplankton.

The biomass of total cells of *Synechococcus* and of pico-eukaryote I contribute to the variation in ligand concentration in the samples below the MDCM (Table 4, Fig. 6 E and F). Especially the relation with pico-eukaryote I is strong ($R^2=0.8$), but it must be kept in mind that the number of samples is very low here ($n=4$). The clusters of data points in Fig. 6 show that although the circumstances are different between the samples above and below the MDCM, the ligand concentrations show comparable relations with phytoplankton variables as total number of cell (6A), total biomass (6C), and the biomass of pico-eukaryote I and II (6F and G). The biomass of *Synechococcus* is the only variable showing a distinctly different relation with L between the samples above and below the MDCM.

Several recent studies suggest evidence of a relationship between phytoplankton and ligand concentrations in the field. Assuming that increased Fe(III) solubility is directly related to the presence of organic Fe-binding ligands, indirect evidence was provided by trends between Chl-*a* and Fe(III) solubility (Takata et al., 2004) and an increase in Fe(III) solubility during a spring bloom (Nakabayashi et al., 2002). Yet, in the present study a direct relationship between organic Fe-binding ligands and phytoplankton cell numbers and biomass could be established.

In general it was not possible to distinguish two ligand classes in the Canary Basin, although the K' values of station 34 were higher then of the other stations. Wells and Trick (2004) acknowledge two classes of natural organic Fe ligands with $K'=10^{22}$ and $K'=10^{21}$. As the concentration and the conditional stability constant K' (Table 1) of the organic Fe-binding ligands likely represent a mixture of ligand groups, higher K' values found in station 34 are probably caused by a relatively high concentration of a strong ligand class.

In conclusion this work confirms the role that phytoplankton plays in the speciation and consequent solubility of Fe. A direct relationship was found between phytoplankton abundance and even species composition and dissolved organic ligand concentration. Our data suggests that not only bacterioplankton influence ligand concentration as observed by others (Reid et al., 1993; Wilhelm and Trick, 1994; Martinez et al., 2001; McCormack et al., 2003; Gledhill et al., 2004) but also eukaryotes with a possible role of diatoms. This confirms earlier suggestions of production of ligands by eukaryotic phytoplankton in the field (Van den Berg, 1995; Croot et al., 2001), yet only found in cultures (Trick et al., 1983; Boye and Van den Berg, 2000). In the Canary Basin at the DCM, the phytoplankton abundance explains 62% of the variation in ligand concentration. The production of these ligands

is probably due to inadvertent lysis instead of actively produced by an inductive mechanism.

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